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Interleukin-36 potently stimulates human M2 macrophages, Langerhans cells and keratinocytes to produce pro-inflammatory cytokines

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ABSTRACT

Interleukin (IL)-36 cytokines belong to the IL-1 family and include three agonists, IL-36 α, β and γ and one inhibitor, IL-36 receptor antagonist (IL-36Ra). IL-36 and IL-1 (α and β) activate similar intracellular pathways via their related heterodimeric receptors, IL-36R/IL-1RAcP and IL-1R1/IL-1RAcP, respectively. However, excessive IL-36 versus IL-1 signaling induces different phenotypes in humans, which may be related to differential expression of their respective receptors.

We examined the expression of IL-36R, IL-1R1 and IL-1RAcP mRNA in human peripheral blood, tonsil and skin immune cells by RT-qPCR. Monocyte-derived dendritic cells (MDDC), M0, M1 or M2-polarized macrophages, primary keratinocytes, dermal macrophages and Langerhans cells (LC) were stimulated with IL-1β or IL-36β. Cytokine production was assessed by RT-qPCR and immunoassays.

The highest levels of IL-36R mRNA were found in skin-derived keratinocytes, LC, dermal macrophages and dermal CD1a$^+$ DC. In the blood and in tonsils, IL-36R mRNA was predominantly found in myeloid cells. By contrast, IL-1R1 mRNA was detected in almost all cell types with higher levels in tonsil and skin compared to peripheral blood immune cells. IL-36β was as potent as IL-1β in stimulating M2 macrophages, keratinocytes and LC, less potent than IL-1β in stimulating M0 macrophages and MDDC, and exerted no effects in M1 and dermal macrophages. Levels of IL-1Ra diminished the ability of M2 macrophages to respond to IL-1.

Taken together, these data are consistent with the association of excessive IL-36 signaling with an inflammatory skin phenotype and identify human LC and M2 macrophages as new IL-36 target cells.

1. Introduction

Interleukin (IL)-36 cytokines belong to the IL-1 family and include three agonists, IL-36α, IL-36β and IL-36γ, and one antagonist, IL-36 receptor antagonist (IL-36Ra). N-terminal processing of these cytokines drastically increases their biological activities and neutrophil proteases were recently shown to catalyze this reaction [1,2]. IL-36 cytokines share many similarities with other IL-1 cytokines. Indeed, the genes encoding IL-36 map on human chromosome 2 like most other IL-1 cytokine genes. IL-36 and IL-1 cytokines share a common C-terminal tertiary structure and their N-terminal sequence is devoid of a leader peptide. IL-1 (α and β)

Abbreviations: ANOVA, analysis of variance; BDCA, blood dendritic cell antigen; DC, dendritic cells; GUSB, gene encoding β-glucuronidase; IFN, interferon; IL-1, interleukin-1; IL-1R1, interleukin-1 receptor 1; IL-1RAcP, interleukin-1 receptor accessory protein; IL-1Ra, interleukin-1 receptor antagonist; IL-36, interleukin-36; IL-36R, interleukin-36 receptor; IL-36Ra, interleukin-36 receptor antagonist; LC, Langerhans cells; LPS, lipopolysaccharides; MAPK, mitogen-activated protein kinase; MDDC, monocyte-derived dendritic cells; NF-κB, nuclear factor-κB; PBMC, peripheral blood mononuclear cells; RT-qPCR, reverse transcription quantitative polymerase chain reaction; S.E.M., standard error of the mean; Th, T helper; TNF, tumor necrosis factor.

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and IL-36 (α, β, and γ) bind to their specific membrane-bound receptors, IL-1R1 or IL-36R, respectively, leading to the recruitment of a common co-receptor (IL-1RAcP) and to the induction of similar intracellular signaling pathways including NF-κB and MAPK [3–7].

IL-36Ra shares 52% amino acid sequence with IL-1Ra, the naturally occurring IL-1 inhibitor, as well as a similar inhibitory mechanism. Both IL-36Ra and IL-1Ra compete with agonists for their receptor binding sites and inhibit their biological activities [2,6,8,9]. A role for IL-36Ra *in vivo* is emphasized by the finding that hereditary IL-36Ra deficiency is associated with the development of generalized pustular psoriasis in humans [10–14] and increased imiquimod-induced psoriasis in mice [15]. On the other hand, IL-1Ra deficiency causes a severe clinical syndrome in humans characterized by systemic inflammation with also skin and bone involvement [16,17]. Concordant with this, IL-1Ra KO mice display increased sensitivity to different models of arthritis and may present spontaneous arthritis or psoriasis-like skin lesions depending on the genetic background [18]. These observations indicate distinct roles for IL-1 and IL-36 despite similar signaling, thus suggesting a different expression pattern of IL-1 and IL-36 ligands and/or receptors.

The expression and biological functions of IL-1 have been well characterized using various *in vitro* and *in vivo* experimental systems. IL-1 exerts potent pro-inflammatory actions by stimulating innate immune cells, Th17 polarization of $CD4^+$ T cells, the expression of adhesion molecules by endothelial cells, and contributes to tissue damage by inducing the production of catabolic enzymes such as matrix metalloproteinases (reviewed in [19]).

In contrast, the expression pattern and functions of IL-36 are less well characterized. IL-36 is mainly expressed in activated epithelia and tonsils [20], but also by macrophages [21,22], dendritic cells (DC), $CD4^+$ T-cells [23–26], plasma cells [27], synovial fibroblasts [28] and keratinocytes [29,30]. *In vitro*-differentiated human and mouse DC express IL-36R and respond to IL-36 by the production of pro-inflammatory cytokines and stimulatory co-receptors [23,25,26]. IL-36R is expressed by mouse naïve $CD4^+$ T cells and IL-36 induces their proliferation, survival and polarization into interferon-γ producing Th1 cells [24]. However, IL-36R signaling is not needed for the control of *Mycobacterial* and *Leishmania* infection *in vivo*, suggesting that it is redundant for Th1 responses to pathogens [31]. Conflicting results were reported regarding IL-36R expression in myeloid DC and monocytes from human blood [25,26]. In mice, the microglia responds to IL-36γ during experimental autoimmune encephalitis [32].

A precise comparison of the relative effects of IL-1 and IL-36 in human immune cells is missing. Furthermore, most of the current knowledge regarding the effects of IL-36 on myeloid cells concerns *in vitro*-derived DC, which may present different characteristics than their *ex vivo* counterparts [33].

We therefore examined the expression of IL-1R1, IL-36R and IL-1RAcP mRNA in human immune cells sorted from the peripheral blood, tonsils and skin. Keratinocytes were used as a positive control throughout the study because they express IL-36R and respond vividly to IL-36 stimulation [29,34]. Our results show that, in addition to keratinocytes, human Langerhans cells (LC), dermal macrophages and dermal $CD1a^+$ DC express higher levels of IL-36R mRNA compared to blood-sorted or blood-derived immune cells and tonsil-sorted immune cells. Most importantly, IL-36β was as potent as IL-1β in stimulating M2-polarized macrophages, keratinocytes and LC, inferior to IL-1β for M0 macrophages and MDDC, and showed no effect neither on M1 nor on dermal macrophages. We further showed that the production of IL-1Ra by M2 macrophages impaired their responsiveness to IL-1β.

2. Material and methods

2.1. Biological reagents

Recombinant processed human IL-36α, IL-36β, IL-36γ [2] and the monoclonal mouse anti-IL-36R neutralizing antibody [5] were generously provided by Amgen Inc. (Seattle, WA, USA). Recombinant human IL-4, GM-CSF, IL-13, IFN-γ and mature IL-1β were purchased from Peprotech (London, UK). The neutralizing monoclonal mouse anti-human IL-1Ra was purchased from R&D Systems (Minneapolis, MN, USA) and ultrapure LPS from InvivoGen (San Diego, CA, USA).

2.2. Cell isolation and culture

Buffy coats from healthy donors were obtained from the Geneva University Hospital Blood Bank after informed consent. Blood was diluted 1:1 with PBS and separated on a Ficoll-Paque PLUS gradient (GE Healthcare, Little Chalfont, UK). The layer containing peripheral blood mononuclear cells (PBMC) was collected and washed twice with PBS. Cells were stained and FACS-sorted as monocytes ($CD20^-$, $CD3^-$, $CD56^-$, $CD14^+$), B cells ($CD20^+$, $CD3^-$, $CD56^-$, $CD14^-$), T cells ($CD20^-$, $CD3^+$, $CD56^-$, $CD14^-$) or NK cells ($CD20^-$, $CD3^-$, $CD56^+$, $CD14^-$) on the Astrios cell-sorter (Beckman Coulter, Brea, CA, USA) and the cell purity was over 90%.

For *in vitro* cell differentiation, monocytes were enriched from PBMC by negative magnetic separation using the monocyte isolation kit II and the AutoMACS pro system (both from Miltenyi Biotech, Bergisch Gladbach, Germany) according to the manufacturer's instructions. The purity of monocytes, assessed by CD14 cell surface expression, was over 85%. In order to generate MDDC, monocytes were resuspended at 6.7×10^5 cells/mL in RPMI glutamax (Thermo Fisher Scientific, Waltham, MA, USA), 10% FCS, 1% non-essential amino acids, 100 U/mL penicillin and 100 μg/mL streptomycin, 1 mM sodium pyruvate, GM-CSF and IL-4 (50 ng/mL each), and plated in culture-treated 6-well plates (Thermo Fisher Scientific) in 3 mL/well. Alternatively, to generate unpolarized macrophages (referred as M0 macrophages), monocytes were resuspended at 2×10^6 cells/mL in X-vivo 10 medium (Lonza, Basel, Switzerland), 2.5% human serum, 100 U/mL penicillin and 100 μg/mL streptomycin, 2 mM L-glutamine, and plated in non-tissue culture-treated 6-well plates (Thermo Fisher Scientific) in 1.5 mL/well. Ultrapure LPS and IFN-γ (20 ng/mL each) or IL-4 and IL-13 (10 ng/mL each) were added at day 0 to the M0 conditions in order to generate M1 or M2-polarized macrophages, respectively. Cytokines were renewed every two days for MDDC and at day 3 and day 7 for macrophages. At day 8, cell purity and differentiation was assessed by flow cytometry. MDDC were over 80% $CD209^+$. M0, M1 or M2-polarized macrophages were over 90% $CD14^{low}$ $CD86^{med}$ $CD200^-$ $CD206^{low}$ $CD80^-$, $CD14^{med}$ $CD86^{low}$ $CD200^-$ $CD206^-$ $CD80^+$ or $CD14^-$ $CD86^{high}$ $CD200^+$ $CD206^{med}$ $CD80^-$, respectively.

Total circulating blood DC (comprising myeloid DC1, myeloid DC2 and plasmacytoid DC) were enriched from PBMC using blood dendritic cell isolation kit II and the AutoMACS pro system (both from Miltenyi) according to manufacturer's instructions. Cell purity, defined using a FSC/SSC linear dot plot and a cocktail of antibodies against CD14, BDCA1, BDCA2 and BDCA3, was over 75%.

For granulocyte isolation, fresh peripheral blood from healthy donors was collected by venous puncture after informed consent. Blood was diluted 1:1 with PBS and separated on a Ficoll-Paque PLUS gradient. The granulocyte layer was collected, resuspended in 1.5% dextran (Sigma, Buchs, Switzerland) in PBS and left 1 h at RT. The upper phase was collected, resuspended in ice cold erythrocyte lysis buffer (155 mM NH_4Cl, 10 mM KH_2CO_3, 0.1 mM

EDTA in ddH_2O) and incubated on ice for 15 min. Cells were then washed once in PBS before further use. Cell purity assessed by CD66b FACS staining was over 95%.

Keratinocytes, LC, dermal macrophages and dermal DC were isolated from human skin samples resulting from cosmetic surgery (abdominoplasty) after informed consent. For the isolation of keratinocytes, the epidermis was peeled off from the dermis after direct incubation of skin fragments in K-SFM medium supplemented with 10 mg/mL dispase (Thermo Fisher Scientific) overnight at 4 °C. The epidermis was then briefly digested with 0.05% tryptase-EDTA (Thermo Fisher Scientific). Cells were resuspended in K-SFM medium containing epidermal growth factor and pituitary extract (all from Thermo Fisher Scientific) and plated in collagen type V-coated dishes (Sigma). Cells were passaged at 80% confluence and collected for further use between passages 1–4.

Alternatively, in order to isolate LC and dermal macrophages, the epidermis was peeled off after direct incubation of skin fragments in trypsin (Merck, Kenilworth, NJ, USA) 0.5% in PBS for one hour at 37°. The dermis was digested with collagenase 1 mg/mL (Thermo Fisher Scientific) and DNAse 33.3 μg/mL (Sigma) in complete medium (RPMI glutamax, 10 mM HEPES, 10% FCS, 100 U/mL penicillin, 100 μg/mL streptomycin, 50 μg/mL Gentamicin) for 18 h at 37 °C [35,36]. The cell suspension was then cultured in complete medium for 48 h at 37 °C. Cells were collected and positively enriched using anti-CD14 magnetic microbeads (Miltenyi), before flow cytometry sorting on a FACSAria II cell-sorter (BD biosciences, San Jose, CA, USA). Sorted dermal macrophages were over 90% $MHCII^{high}$ $CD14^{high}$ $CD86^{low}$. The epidermal fraction was briefly treated with DNAse 0.17 mg/mL in RPMI at 37 °C. The LC in the epidermal suspension were then enriched by Ficoll-Paque gradient separation and by positive selection with anti-CD1a magnetic microbeads (Miltenyi) before flow cytometry sorting. LC were over 90% $MHCII^{high}$ $CD1a^{+}$ Langerin/$CD207^{+}$.

In order to isolate emigrating dermal DC, ~2 cm^2 skin pieces were placed on 40 μm cell strainers laid on complete medium in 6-well culture-treated plates (Thermo Fisher Scientific) for 4 days [37]. Emigrating cells were then collected from the supernatant, stained and sorted with over 90% purity as dermal $CD1a^{+}$ DCs ($MHCII^{high}$ $CD14^{-}$ Langerin/$CD207^{-}$) or dermal $CD14^{+}$ DCs ($MHCII^{int}$ $CD14^{+}$ Langerin/$CD207^{-}$).

Tonsils were obtained from routine tonsillectomies performed at the Ospedale San Giovanni (Bellinzona, Switzerland) after informed consent. After mincing, tonsils were treated with 1 mg/mL collagenase D and 1 mg/mL DNAse (Roche Diagnostics, Meylan, France) for 60 min at 37 °C. Tonsillar mononuclear cells were prepared by Ficoll-Paque density centrifugation and then FACS-sorted on a FACSAria III (Becton Dickinson, Franklin Lakes, NJ, USA) with a purity over 97%. B cells were sorted as $CD19^{+}$ $CD3^{-}$, CD4 T cells as $CD19^{-}$ $CD3^{+}$ $CD4^{+}$ $CD8^{-}$, CD8 T cells as $CD19^{-}$ $CD3^{+}$ $CD4^{-}$ $CD8^{+}$ $CD56^{-}$, NK cells as $CD19^{-}$ $CD3^{-}$ $CD4^{-}$ $CD8^{-}$ $CD56^{+}$, pDC as $CD3^{-}$ $CD19^{-}$ $BDCA4^{+}$ $CD11c^{-}$, myeloid DC as $CD3^{-}$ $CD19^{-}$ $BDCA4^{-}$ $CD11c^{+}$ $CD14^{-}$ and monocytes as $CD3^{-}$ $CD19^{-}$ $BDCA4^{-}$ $CD11c^{+}$ $CD14^{+}$.

2.3. Stimulation of MDDC, polarized macrophages and skin-sorted cells

After eight days of differentiation, MDDC were detached by pipetting, pooled and plated in fresh differentiation medium at the concentration indicated in the figure legends. Mature IL-1β or processed IL-36 were then added with or without pre-incubation with the anti-IL-36R neutralizing antibody or an adequate isotype control antibody (mouse IgG1 anti-GP3-peptibody, Amgen) at concentrations indicated in the figure legends. At various time-points, supernatants were collected and cells were lyzed in Trizol reagent (Thermo Fisher Scientific) for RNA processing.

Polarized macrophages were stimulated by the addition of 100 ng/mL of mature IL-1β or processed IL-36β, either directly in the differentiation culture without renewing medium or after de novo plating as indicated in the figure legends. Optionally, the anti-IL-1Ra neutralizing antibody was added at the time of stimulation. At various time-points, supernatants were collected and cells lyzed in Trizol reagent.

Primary human keratinocytes were detached using 0.05% tryptase-EDTA and plated in K-SFM medium at 7×10^4 cells/mL. LC and dermal macrophages were suspended at 5×10^4 and 5×10^5 cells/mL in complete medium, respectively. Mature IL-1β or processed IL-36β (100 ng/mL each) were then added and supernatants were collected 24 h later.

2.4. RNA isolation and quantitative reverse transcription PCR (RT-qPCR)

Total RNA was extracted using Trizol reagent and treated with DNase RQ1 (Promega, Fitchburg, WI, USA) according to manufacturer's instructions. Reverse transcription was performed using superscript II reverse transcriptase (Thermo Fisher Scientific). Messenger RNA levels of target genes were assessed by quantitative PCR using SYBR green PCR mastermix (Thermo Fisher Scientific) and specific primer pairs (Eurofins Genomics, Ebersberg, Germany) on a Stepone Plus Real-time PCR system (Thermo Fisher Scientific). The $2^{-\Delta Ct}$ method was used to determine the relative expression of the target genes to the housekeeping gene GUSB. Each primer pair was separated by at least one intron on the relevant genbank sequences. The following primer sequences (5′→3′) were used: IL-36R Fw-gctcccagctccggattttc Rev-gtggggcttggggtataagac, GUSB Fw-ccaccagggaccatccaat Rev-agtcaaaatatgtgttctggacaaagtaa, IL-1R1 Fw-cctccaggattcatcaacac Rev-aaaactccatataagggcacac, IL-1RAcP Fw-gaccctccgtggagttttggagaa Rev-tagaacaaccaggaggcgtct, IL-6 Fw-tgtagccgccccacacagaca Rev-ctgccagtgcctctttgctgct, TNF-α Fw-agtgatcggccccagaggga Rev-actggagctgcccctcagcttg, IL-12p35 Fw-gctccagaaggccagacaaa Rev-ggccaggcaactcccattag, IL-12p40 Fw-agggacatcatcaaacctgacc Rev-gctgaggtcttgtccgtgaa, IL-23p19 Fw-gtgggacacatggatctaagagaa Rev-tcagaccctggtggatcctt, IL-8 Fw-caccggaaggaaccatctcact Rev-tgcaccttcacacagagctgc, IL-10 Fw-gcctaacatgcttcgagatc Rev-tgatgtctgggtcttggttc.

2.5. Taqman Low Density Array (TLDA)

For Supplementary Fig. 3, total RNA was extracted using the RNeasy Mini kit (Qiagen, Valencia, CA), and cDNA synthesis was accomplished using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Custom designed TLDA with 48 query genes and 2 control genes (HPRT and 18S rRNA) were performed on an 7900HT Real-Time PCR System (Plate and System from Applied Biosystems).

2.6. Immunoassays

Supernatants were tested for IL-6, IL-8 (human ELISA Ready-SET Go!, ebioscience, San Diego, CA, USA) and IL-1Ra (human IL-1Ra Duoset ELISA kit, R&D Systems) levels by ELISA. Alternatively, IL-6, IL-8, IL-12p70, IL-23, TNF-α and IL-10 levels were measured by multiplex assay using the Bioplex Magpix Multiplex reader and customized microbead and antibody sets (Biorad, Hercules, CA, USA). For Supplementary Fig. 3, the Human Proinflammatory II 4-plex kit (MSD, Rockville, Maryland) was used to determine levels of IL-1β, IL-8 and TNF-α.

2.7. Flow cytometry analysis

Cells were collected by pipetting or gentle scratching and washed once in FACS buffer (1% BSA, 10 mM EDTA in PBS). Fc receptors were blocked by incubation of 0.25×10^6 to 2×10^6 - cells in 50 µL of 10% human serum in FACS buffer for 15 min at 4 °C. Cells were then labeled with a cocktail of antibodies diluted in FACS buffer in a total volume of 50 µL for 30 min at 4 °C. Cell viability was assessed by staining with Zombie Yellow (eBioscience) according to manufacturer's instructions. Data were acquired on a Gallios 4 flow cytometer (Beckman Coulter). One comp ebeads (ebioscience) were used for compensation. Using Kaluza software, total cells were gated on FSC/SSC area linear plots and doublets were excluded using FSC height/FSC area linear plots. Dead cells were counted and excluded using Zombie Yellow histograms and cell purity and the phenotype was determined using the specific markers described in the cell isolation and culture part.

HLA-DR-FITC, CD1a-FITC, CD1a-APC, CD11c-APC, CD14-FITC, CD20-APC, CD80-V450, CD86-FITC, CD200-Per-CP-Cy5.5, CD206-APC and CD209-Per-CP-Cy5.5 were from BD biosciences. HLA-DR-PE-Cy7, CD3-FITC, CD3-PE-Cy5 and CD56-PE-Cy5 were from Beckman-Coulter. BDCA-1-APC, BDCA-2-PE, BDCA-3-ViobrightFITC and BDCA4-PE were from Miltenyi. CD3-PE, CD19-PE-Cy7, CD66b-PE-Cy7 and CD86-PE were from ebiosciences. CD4-BV421, CD56-BV421 and Zombie Yellow were from Biolegend (San Diego, CA, USA). CD8-PE-TxRED was from Thermo Fisher Scientific. Langerin/CD207-PE was from Dendritics (Lyon, France) and CD14-PE from ImmunoTools (Friesoythe, Germany). For cell sorting of skin cells, dead cells were excluded as DAPI-positive.

2.8. Statistical analysis

Unless otherwise stated in the figure legend, $n \geqslant 3$ and Tukey boxplots are shown. Assuming a normal distribution, paired one-way or two-way ANOVA were performed depending on the experimental conditions. P-values are shown.

3. Results

3.1. IL-36R mRNA is expressed in blood-sorted and blood-derived human myeloid cells, though at low levels compared to keratinocytes

In order to examine the relative responsiveness of human immune cells to IL-36 and IL-1, we first investigated the expression profile of IL-36R (Fig. 1A), IL-1R1 (1B) and IL-1RAcP (1C) mRNA in monocytes, circulating DC, granulocytes, B-cells, T-cells, NK cells and PBMC isolated from the peripheral blood of healthy donors, as well as in *in vitro* differentiated MDDC and M0-, M1-, and M2-polarized macrophages, while primary keratinocytes served as a positive control. The expression of IL-36R mRNA was restricted to the myeloid lineage, however with much lower levels compared to keratinocytes (~100-fold). In the myeloid lineage, M0 and M2 macrophages, MDDC and granulocytes expressed higher levels than the other tested cells. IL-1R1 mRNA was found in all cell types with the exception of B-cells, with noticeably higher levels in keratinocytes, M0 and M1 macrophages, MDDC and granulocytes. IL-1RAcP mRNA was ubiquitously expressed, although at higher levels in keratinocytes, MDDC and granulocytes.

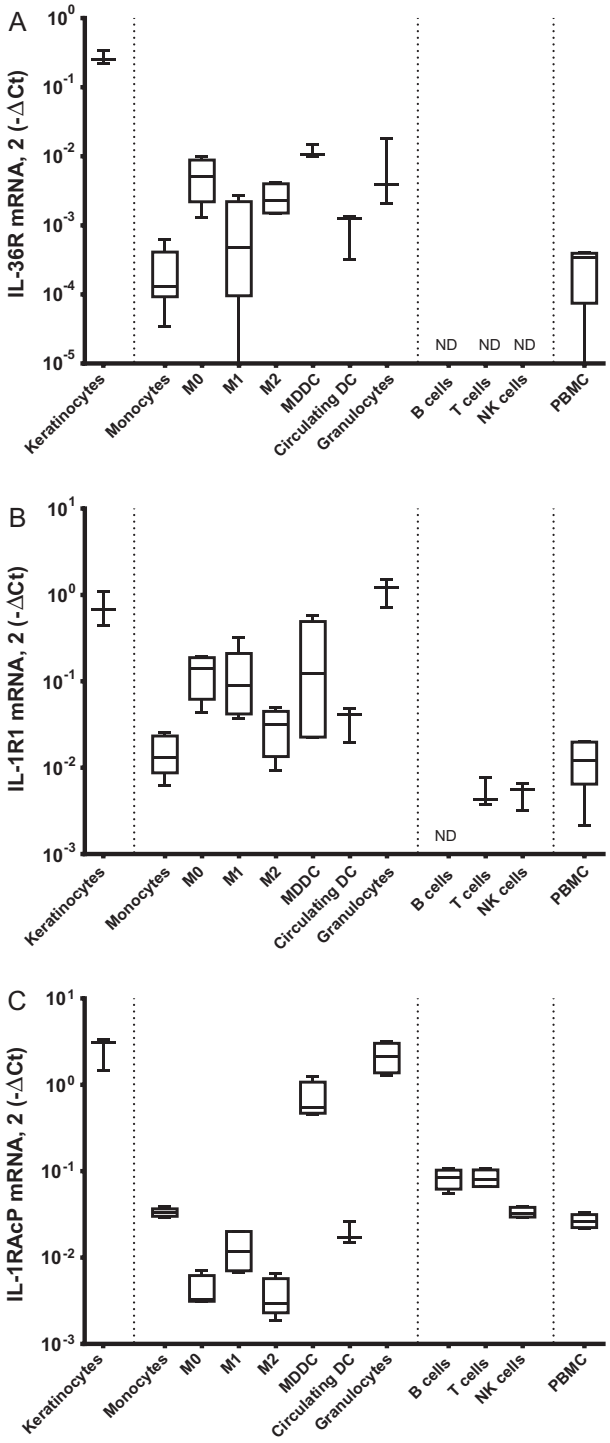

Fig. 1. Messenger RNA levels of IL-36R, IL-1R1 and IL-1RAcP in human keratinocytes, *ex-vivo* differentiated macrophages and dendritic cells and in blood-sorted cells. Cells were isolated and cultured as described in Section 2. Total RNA was extracted and RT-qPCR for IL-36R (A), IL-1R1 (B) and IL-1RAcP (C) were performed using a Stepone plus Real-Time PCR system. Data were normalized against the housekeeping gene GUSB. Tukey boxplots are represented. ND = all samples Not Detected.

3.2. IL-36 stimulates human MDDC to a lesser extent than IL-1

In light of the expression profile of IL-36R, IL-1R1, and their co-receptor IL-1RAcP mRNA in human MDDC, the stimulatory effects of IL-36 and IL-1 were first compared in these cells. Dose-response and time course experiments were performed to select the optimal conditions (Supplementary Fig. 1A, B and C). All three IL-36 isoforms induced IL-6 production in a similar manner, with maximal mRNA and protein levels observed after 6 h and 24 h of

stimulation, respectively. The dose-response curve reached a plateau at 100 ng/mL. The stimulatory effect of IL-36 was abrogated when cells were pre-incubated with a neutralizing anti-IL-36R antibody (Supplementary Fig. 1D).

Therefore, MDDC were subsequently stimulated with 100 ng/mL of IL-36β or IL-1β, and levels of IL-6, TNF-α, IL-8, IL-12p70, IL-23 and IL-10 mRNA and proteins were measured by RT-qPCR (Supplementary Fig. 2) or multiplex and ELISA (Fig. 2) at 6 and 24 h, respectively. The results showed that IL-36β significantly induced the production of some of these cytokines, but the response was much lower than that induced by IL-1β (10- to 100-fold).

3.3. IL-36β is as potent as IL-1β in inducing cytokine production by human M2 macrophages but not by M0 and M1 macrophages

Given their differential expression of IL-36R and IL-1R1 mRNA, we next focused on human monocyte-derived M0, M1 and M2 macrophages. After confirming their polarization status by flow cytometry (Fig. 3A), the cells were stimulated with IL-36β or IL-1β (100 ng/mL each) for 24 h and IL-6 levels were measured in the supernatant by ELISA (Fig. 3B). Both IL-1β and IL-36β induced the production of IL-6 by M0 and M2 macrophages. IL-36β appeared slightly more potent than IL-1β in stimulating M2 macrophages, whereas IL-1β exerted stronger stimulatory effects on M0

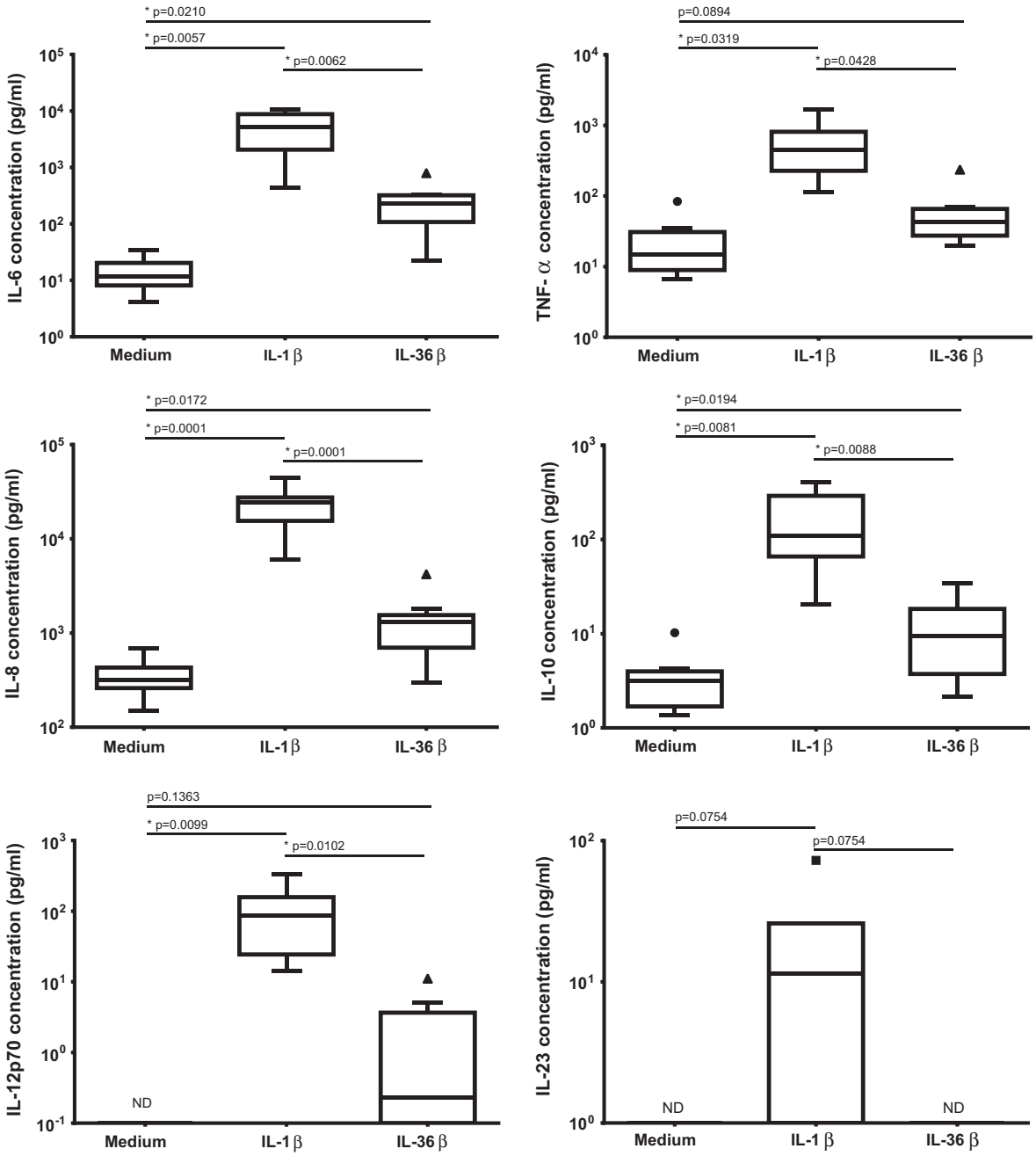

Fig. 2. Comparative effects of IL-1β and IL-36β on human monocyte-derived dendritic cells. Monocyte-derived dendritic cells (MDDC) were obtained as described in Section 2. At day 8, MDDC were resuspended at 5×10^5 cells/mL in differentiation medium and cultured with or without IL-1β or IL-36β (100 ng/mL each) for 24 h. Cytokine and chemokine protein levels in cell supernatants were determined by Biorad multiplex assay or ELISA (IL-8). Tukey boxplots are represented. Paired one-way ANOVA were performed and p-values are shown.

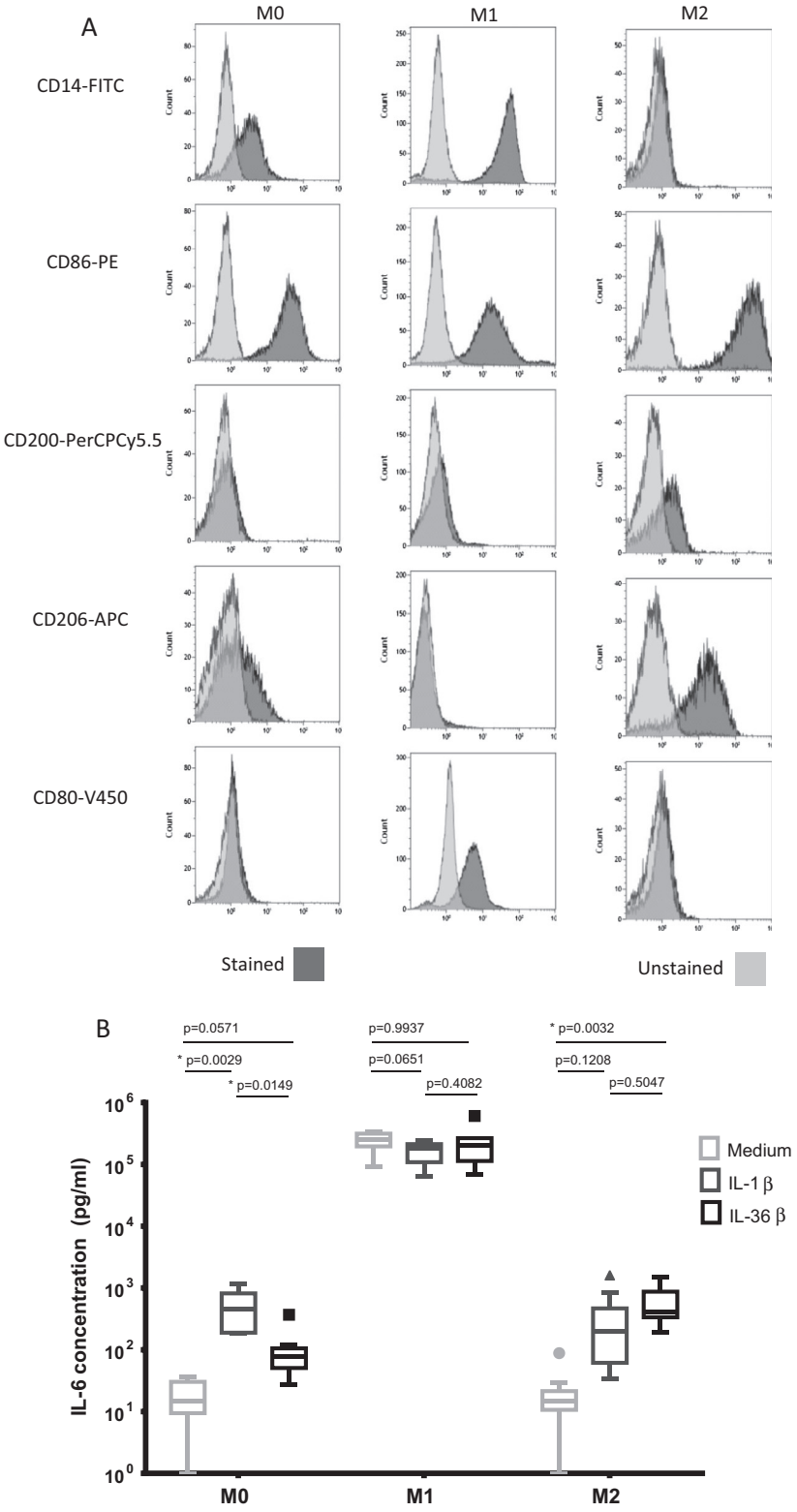

Fig. 3. Comparative effects of IL-1β and IL-36β on human polarized macrophages. Polarized macrophages were obtained as described in Section 2. At day 8, macrophages were collected and stained (dark) with a cocktail of antibodies or left untreated (light) before FACS-analysis was performed to assess the polarization (A). One representative example out of ten is shown. Macrophages were stimulated in the differentiation medium by the addition of IL-1β or IL-36β (100 ng/mL each). After 24 h, cell supernatants were collected and IL-6 levels were measured by ELISA (B). Tukey boxplots are represented. Paired one-way ANOVA were performed on M0, M1 and M2 subgroups and p-values are shown.

macrophages. Of note, IL-36β induced the production of pro-inflammatory mediators (IL-1β, IL-8, TNF-α) by M2 macrophages (Supplementary Fig. 3). Because M1 polarization conditions include LPS and IFN-γ, supernatants of unstimulated M1 macrophages contained high levels of IL-6, that neither IL-1β nor IL-36β were able to further increase. Macrophage activation was therefore

repeated after medium replacement (Supplementary Fig. 4). Under these culture conditions the stimulatory effects of IL-1β tended to increase in M2 macrophages, suggesting the presence of an IL-1β inhibitor in the supernatant of M2 macrophages. Conversely, the stimulatory effect of IL-1β was visible in M1 macrophages, whereas these cells remained unresponsive to IL-36β stimulation.

3.4. IL-1Ra impairs the response of human M2 macrophages to IL-1β

We hypothesized that the impaired response of human M2 macrophages to IL-1β seen in Fig. 3B could be related to the presence of high levels of IL-1Ra in the cell supernatant. Indeed, elevated IL-1Ra levels were found in M2 macrophage cultures as compared to M0- and M1-polarized macrophage cultures (Fig. 4A). We therefore, stimulated M2 macrophages with IL-1β or IL-36β (100 ng/mL each) in the absence or presence of a neutralizing anti-IL-1Ra antibody. As depicted in Fig. 4B, the stimulatory effect of IL-1β, but not of IL-36β, was significantly increased upon IL-1Ra blockade.

3.5. IL-36R mRNA is detected in human myeloid DC, monocytes and CD4$^+$ T-cells sorted from tonsils

To extend our study to human immune cells of secondary lymphoid organs, we isolated different cell types from human tonsils to high purity and determined the expression of IL-36R (Fig. 5A), IL-1R1 (5B) and IL-1RAcP (5C) mRNA by RT-qPCR. Again, skin-isolated keratinocytes were used as positive control. The expression of IL-36R mRNA was detected in the myeloid lineage (monocytes and mDC) but at 10-fold lower levels compared to keratinocytes. However, as opposed to results obtained in

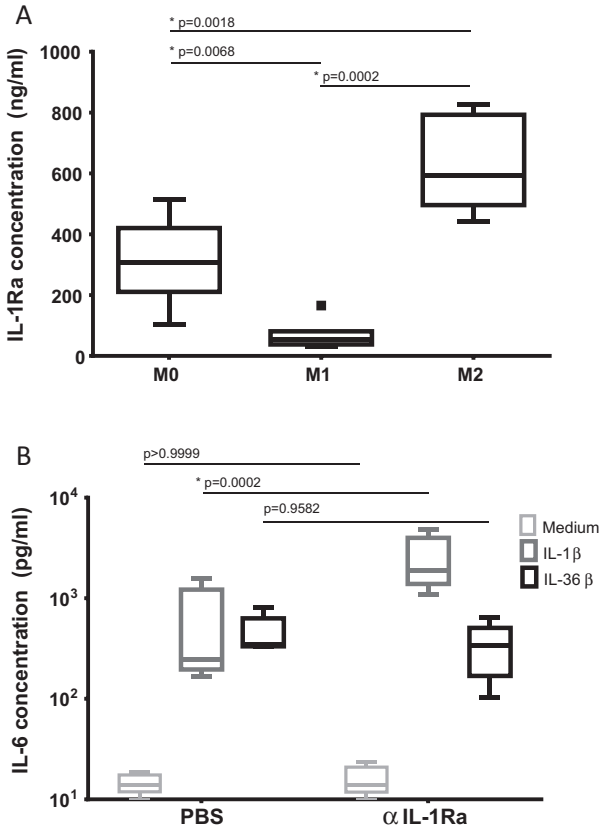

Fig. 4. IL-1Ra impairs the response of human M2 macrophages to IL-1β. Polarized macrophages were generated as described in Section 2. On day 10, supernatants were collected and IL-1Ra levels were measured by ELISA (A). (B) M2 macrophages were stimulated with IL-1β or IL-36β (100 ng/mL each) in presence of a neutralizing anti-IL-1Ra antibody 20 μg/mL or PBS. After 24 h, cell supernatants were collected and IL-6 levels were measured by ELISA. Tukey boxplots are represented. Paired one-way ANOVA (A) and paired two-way ANOVA (B) were performed and respective p-values are shown.

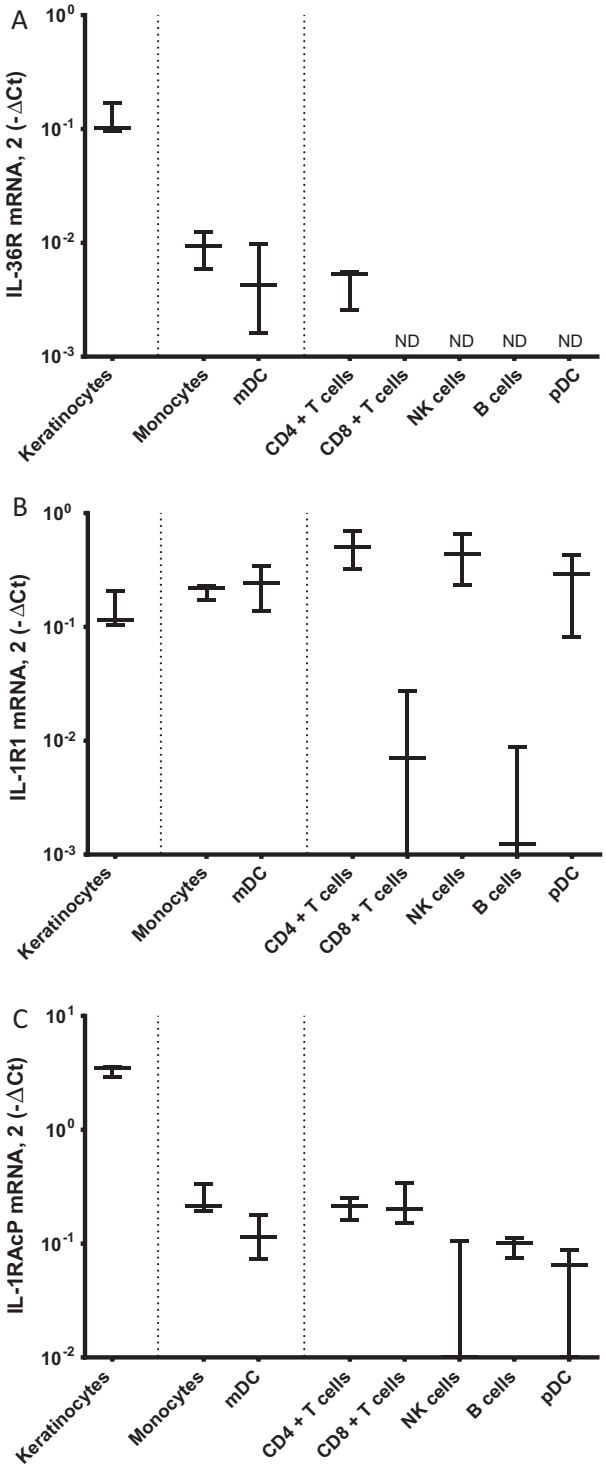

Fig. 5. Messenger RNA levels of IL-36R, IL-1R1 and IL-1RAcP in human tonsil-sorted immune cells and in keratinocytes. Cells from tonsils were isolated as described in Section 2. Total RNA was extracted and RT-qPCR for IL-36R (A), IL-1R1 (B) and IL-1RAcP (C) were performed using a Stepone plus Real-Time PCR system. Data were normalized against the housekeeping gene GUSB. Tukey boxplots are represented. ND = all samples Not Detected.

blood where total T-cells did not express IL-36R mRNA, tonsil CD4$^+$ T-cells expressed IL-36R mRNA. IL-1R1 mRNA was detected in all cell types at similar levels with the exception of B-cells and CD8$^+$ T-cells that displayed lower transcriptional activity. IL-1RAcP mRNA was expressed in all cell types with lower levels in NK cells.

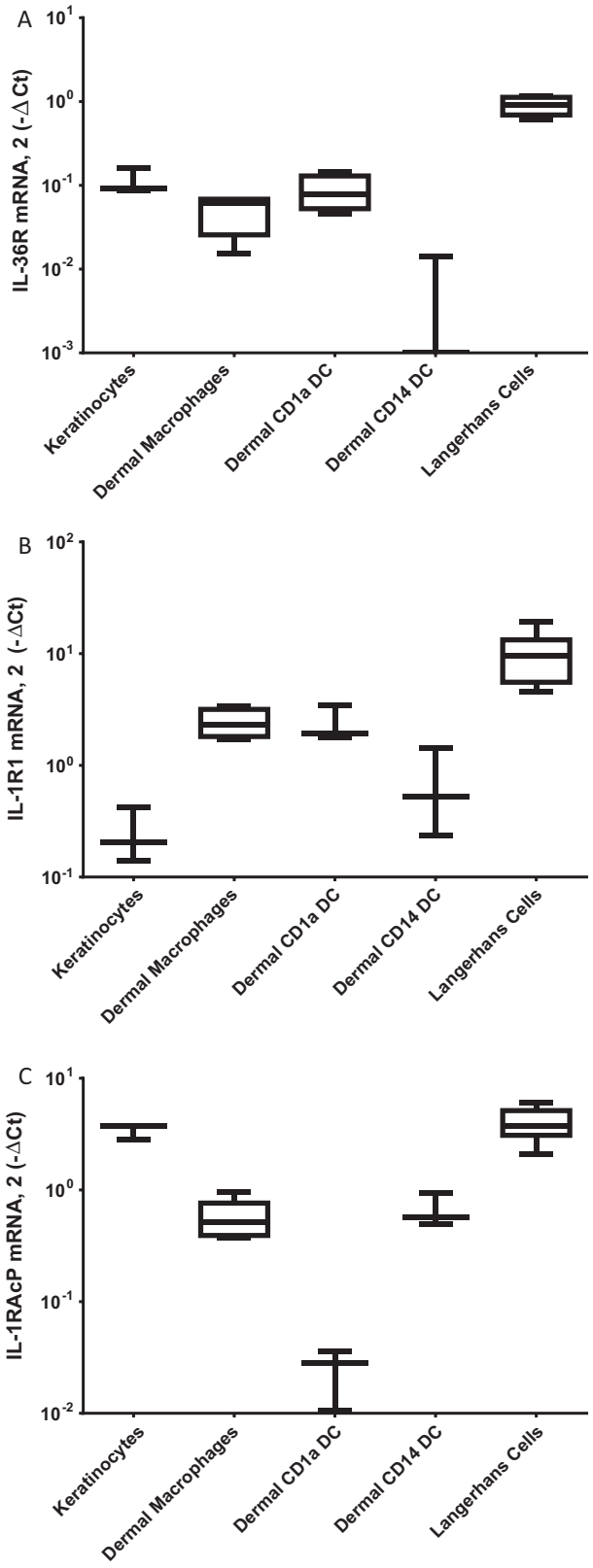

Fig. 6. Messenger RNA levels of IL-36R, IL-1R1 and IL-1RAcP in human skin-sorted cells and in keratinocytes. Cells from the skin were isolated as described in Section 2. Total RNA was extracted and RT-qPCR for IL-36R (A), IL-1R1 (B) and IL-1RAcP (C) were performed using a Stepone plus Real-Time PCR system. Data were normalized against the housekeeping gene GUSB. Tukey boxplots are represented.

3.6. IL-36R mRNA is expressed at high levels in human skin immune cells

Given the known role of IL-36 in skin inflammation [6,10,11,15,29,30,34,38–42], we then examined the levels of IL-36R (Fig. 6A), IL-1R1 (6B) and IL-1RAcP (6C) mRNA in human immune cells isolated from the skin, again in comparison with primary keratinocytes. As opposed to our findings with tonsil and blood purified cells, IL-36R mRNA levels of dermal macrophages, dermal CD1a$^+$ DC were as high as those in keratinocytes. Even more striking, the IL-36R mRNA levels were higher in human LC. In contrast, dermal CD14$^+$ DC exhibited relatively low IL-36R mRNA levels. IL-1R1 mRNA was detected in all cell types with LC again expressing the highest levels. IL-1RAcP was ubiquitously expressed, with dermal CD1a$^+$ DC expressing much lower levels than the other cell types.

3.7. IL-1 and IL-36 exert similar effects on human keratinocytes and LC, but not on dermal macrophages

In order to investigate their relative responses to IL-36 and IL-1, human LC, keratinocytes, and dermal macrophages were stimulated with IL-1β or IL-36β. IL-36β appeared to be as potent as IL-1β in inducing cytokine production by LC (Fig. 7A) and keratinocytes (Supplementary Fig. 5), whereas dermal macrophages responded more potently to IL-1β stimulation (7B).

4. Discussion

In the present study, we determined the expression levels of IL-36R, IL-1R1 and IL-1RAcP by RT-qPCR in a variety of human immune cells sorted or *ex vivo* differentiated from the peripheral blood, as well as sorted from tonsils or the skin. The highest levels of IL-36R mRNA were found in skin immune cells, particularly in LC, and in keratinocytes. In blood and tonsils, the expression of IL-36R was mainly present in myeloid cells. By contrast, IL-1R1 mRNA was detected in almost all cell types tested, with higher levels in tonsils and skin immune cells. IL-36β was as potent as IL-1β in stimulating M2 macrophages, keratinocytes and LC, less potent than IL-1β in stimulating M0 macrophages and MDDC, and exerted no effects in M1 and dermal macrophages. IL-1Ra production by M2 macrophages impaired their responsiveness to IL-1β.

Several findings indicate that IL-36 is involved in skin inflammation [38]. Indeed, all IL-36 isoforms are overexpressed in psoriatic skin, including in psoriasis-like skin lesions secondary to anti-TNF treatment [6,42]. Moreover, IL-36 induces several pro-inflammatory mediators in human keratinocytes *in vitro* and is itself induced in keratinocytes stimulated by TNF-α, IL-17, IL-22, IFN-γ or Poly(I;C) [29,30,34]. Transgenic mice overexpressing IL-36α in keratinocytes develop psoriasis-like lesions with acanthosis, parakeratosis, and elevated levels of psoriasis-associated cytokines [40]. The imiquimod-induced mouse model of psoriasis is completely dependent on IL-36 and more specifically on IL-36α [15,41]. Immunodeficient mice engrafted with human psoriatic skin and treated with anti-IL-36R antibodies present with a very limited pathology [39]. Our results, showing that human skin antigen-presenting cells express high levels of IL-36R and that human LC and keratinocytes are highly responsive to IL-36 stimulation, further supports a contribution of IL-36 to skin inflammation.

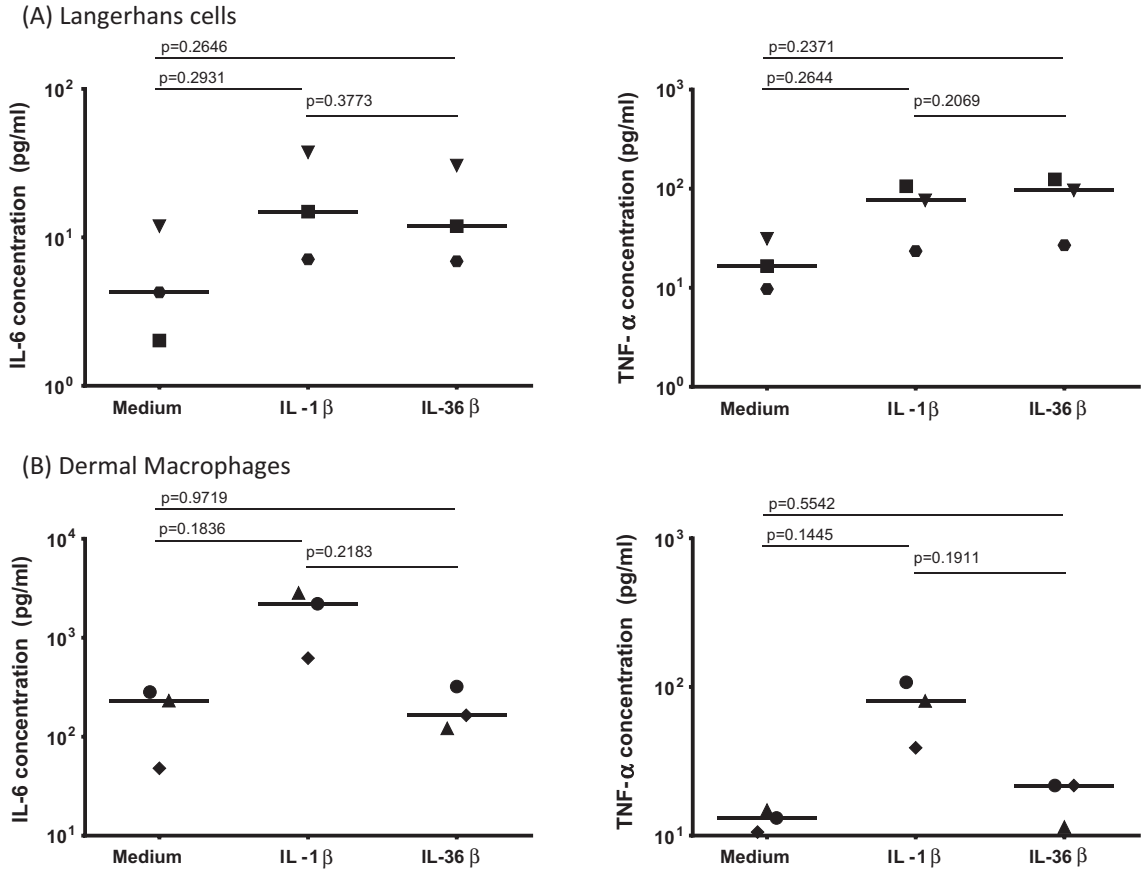

Fig. 7. Comparative effects of IL-1β and IL-36β on human Langerhans cells and dermal macrophages. Langerhans cells (A) and dermal macrophages (B) were obtained and cultured as described in Section 2 with or without IL-1β or IL-36β (both 100 ng/mL) for 24 h. Cell supernatants were harvested and cytokine protein levels were measured by the Biorad multiplex assay. Each point represents one donor and bars represent the median values. A different symbol was attributed to each donor. Paired one-way ANOVA were performed and p-values are shown.

Peripheral blood human T cells did not express IL-36R mRNA, in line with a previous publication [26]. However, we demonstrate for the first time that IL-36R is expressed by human $CD4^+$ (but not $CD8^+$) T cells from tonsils. This difference may be explained by the local microenvironment and warrant further investigations.

An interesting characteristic of macrophages is their ability to "switch" from a phenotype to another *in vitro* and *in vivo* [43–45], suggesting that a given cell may participate sequentially in both the induction and the resolution of inflammation [46]. These functions are attributed to specific macrophage activation states [47], including the pro-inflammatory M1 (or classically activated Macrophages, induced by LPS + IFN-γ) and the anti-inflammatory M2 (or alternatively activated Macrophages, induced by IL-4) states. In our study, levels of IL-36R mRNA were higher in human M2 than in M1 macrophages, whereas a reverse pattern was observed for IL-1R1, suggesting that M2 macrophages may be a potential target cell for IL-36 stimulation. Indeed, we observed that IL-36β was slightly more potent than IL-1β in inducing IL-6 production by M2 but not by M1 or M0 macrophages. Moreover, IL-36β was also able to stimulate M2 macrophages to produce IL-1β, IL-8 and TNF-α, turning these cells towards a more pro-inflammatory state. Interestingly, *in vitro* polarized M2 macrophages express also high levels of IL-1Ra leading to attenuated IL-1β responses and potentially with an anti-inflammatory phenotype.

It should be emphasized that in addition to immune cells, many other cell types, including stromal cells and endothelial cells could contribute to the differences in clinical manifestations associated with excessive IL-1 and IL-36 signaling in humans. In a previous study, we compared the effects of IL-1β and IL-36β in human synovial fibroblasts and observed that IL-1β was a much more potent inducer of cytokine responses than IL-36β (GP, CG, unpublished data). Consistent with these findings, IL-1 plays a critical role in several experimental models of arthritis, whereas IL-36 has no effect [48].

One limit to our study is that levels of IL-36R and IL-1R1 mRNA in a particular cell-type are not predictive of its relative response to IL-36 and IL-1. Indeed, dermal macrophages express high levels of both IL-36R and IL-1R1 mRNA, but respond only to IL-1β. The same was observed for granulocytes (data not shown). It is plausible that IL-36 mRNA levels do not properly reflect cell surface receptor levels, thus explaining this discrepancy. Unfortunately, we were unable to confirm this hypothesis because, in our hands, the presence of endogenous IL-36R in human primary cells was not reliably detectable by flow cytometry. Thus, our findings indicate that there is only a partial correlation between the mRNA levels of IL-36R and IL-1R1 and cell responses to IL-36 and IL-1. Another hypothesis to explain the lack of responsiveness to IL-36 could be related to elevated levels of IL-36Ra in cell supernatants. However, this appears unlikely in the case of dermal macrophages and granulocytes, because medium renewal was performed prior stimulation. Finally, the absence of IL-1RAcP or downstream signaling molecules is unlikely since it would theoretically impact the effects of both IL-1 and IL-36.

Quantitative differences may exist in the stimulatory effects elicited by IL-36α, IL-36β and IL-36γ. In Supplementary Fig. 1, we have shown that all IL-36 agonists are equipotent in inducing IL-6 production by MDDC. Regarding the other cell types, IL-36β

was recently shown to be at least as potent as IL-36α and IL-36γ in inducing cytokine production by monocytes and in maturating mDC [26]. Concerning skin cells, IL-36β was shown in different studies to be the most effective IL-36 agonist in inducing the production of cytokines, chemokines and metalloproteinases by human keratinocytes [29,34] and epidermal cell cultures [34]. Finally, IL-36β is at least as produced as the other IL-36 agonists by human keratinocytes stimulated with various pro-inflammatory stimuli [29]. Taken together, these results support the use of IL-36β in our experiments, notably in comparison with IL-1β.

In conclusion, our data further support the role of IL-36 in skin inflammation and have identified human LC as a new IL-36 skin target cell in addition to keratinocytes. Moreover, we have shown for the first time that IL-36 can act on human macrophages, particularly M2 macrophages, and can drive them to a proinflammatory phenotype. We also showed that human macrophage subsets respond differently to IL-1 and IL-36 stimulation.

Authorship

DD designed and performed the experiments, analyzed the data and wrote the manuscript.

PM supervised the project, contributed to the design and the analysis of the experiments and wrote the manuscript.

VF designed, performed and analyzed the experiments involving skin cells.

YS designed, co-performed and co-analyzed the experiments involving M0, M1 and M2 macrophages.

HA and JT designed and co-analyzed the experiments involving M0, M1 and M2 macrophages.

DJ designed and performed the tonsil cell sorting.

NB designed and performed the experiments involving primary human keratinocytes.

CM reviewed the manuscript.

GP supervised the project.

CG supervised the project and wrote the manuscript.

Conflict of interest

YS, HA and JT were employees and stockholders of Amgen during the time that the work was completed. JT is now an employee of Janssen R&D and owns Janssen stock.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cyto.2016.05.012.

References

[1] C.M. Henry, G.P. Sullivan, D.M. Clancy, I.S. Afonina, D. Kulms, S.J. Martin, Neutrophil-derived proteases escalate inflammation through activation of IL-36 family cytokines, Cell Rep. 14 (2016) 708–722.
[2] J.E. Towne, B.R. Renshaw, J. Douangpanya, B.P. Lipsky, M. Shen, C.A. Gabel, et al., Interleukin-36 (IL-36) ligands require processing for full agonist (IL-36alpha, IL-36beta, and IL-36gamma) or antagonist (IL-36Ra) activity, J. Biol. Chem. 286 (2011) 42594–42602.
[3] C. Garlanda, C.A. Dinarello, A. Mantovani, The interleukin-1 family: back to the future, Immunity 39 (2013) 1003–1018.
[4] J.E. Sims, D.E. Smith, The IL-1 family: regulators of immunity, Nat. Rev. Immunol. 10 (2010) 89–102.
[5] J.E. Towne, K.E. Garka, B.R. Renshaw, G.D. Virca, J.E. Sims, Interleukin (IL)-1F6, IL-1F8, and IL-1F9 signal through IL-1Rrp2 and IL-1RAcP to activate the pathway leading to NF-kappaB and MAPKs, J. Biol. Chem. 279 (2004) 13677–13688.
[6] R. Debets, J.C. Timans, B. Homey, S. Zurawski, T.R. Sana, S. Lo, et al., Two novel IL-1 family members, IL-1 delta and IL-1 epsilon, function as an antagonist and agonist of NF-kappa B activation through the orphan IL-1 receptor-related protein 2, J. Immunol. 167 (2001) 1440–1446.
[7] J.E. Sims, C.J. March, D. Cosman, M.B. Widmer, H.R. MacDonald, C.J. McMahan, et al., cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily, Science 241 (1988) 585–589.
[8] W.P. Arend, F.G. Joslin, R.J. Massoni, Effects of immune complexes on production by human monocytes of interleukin 1 or an interleukin 1 inhibitor, J. Immunol. 134 (1985) 3868–3875.
[9] J.F. Balavoine, B. de Rochemonteix, K. Williamson, P. Seckinger, A. Cruchaud, J. M. Dayer, Prostaglandin E2 and collagenase production by fibroblasts and synovial cells is regulated by urine-derived human interleukin 1 and inhibitor (s), J. Clin. Invest. 78 (1986) 1120–1124.
[10] S. Marrakchi, P. Guigue, B.R. Renshaw, A. Puel, X.Y. Pei, S. Fraitag, et al., Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis, N. Engl. J. Med. 365 (2011) 620–628.
[11] A. Onoufriadis, M.A. Simpson, A.E. Pink, P. Di Meglio, C.H. Smith, V. Pullabhatla, et al., Mutations in IL36RN/IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis, Am. J. Hum. Genet. 89 (2011) 432–437.
[12] M. Farooq, H. Nakai, A. Fujimoto, H. Fujikawa, A. Matsuyama, N. Kariya, et al., Mutation analysis of the IL36RN gene in 14 Japanese patients with generalized pustular psoriasis, Hum. Mutat. 34 (2013) 176–183.
[13] K. Sugiura, A. Takemoto, M. Yamaguchi, H. Takahashi, Y. Shoda, T. Mitsuma, et al., The majority of generalized pustular psoriasis without psoriasis vulgaris is caused by deficiency of interleukin-36 receptor antagonist, J. Invest. Dermatol. 133 (2013) 2514–2521.
[14] L. Rossi-Semerano, M. Piram, C. Chiaverini, D. De Ricaud, A. Smahi, I. Kone-Paut, First clinical description of an infant with interleukin-36-receptor antagonist deficiency successfully treated with anakinra, Pediatrics 132 (2013) e1043–e1047.
[15] L. Tortola, E. Rosenwald, B. Abel, H. Blumberg, M. Schafer, A.J. Coyle, et al., Psoriasiform dermatitis is driven by IL-36-mediated DC-keratinocyte crosstalk, J. Clin. Invest. 122 (2012) 3965–3976.
[16] S. Reddy, S. Jia, R. Geoffrey, R. Lorier, M. Suchi, U. Broeckel, et al., An autoinflammatory disease due to homozygous deletion of the IL1RN locus, N. Engl. J. Med. 360 (2009) 2438–2444.
[17] I. Aksentijevich, S.L. Masters, P.J. Ferguson, P. Dancey, J. Frenkel, A. van Royen-Kerkhoff, et al., An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist, N. Engl. J. Med. 360 (2009) 2426–2437.
[18] J. Palomo, D. Dietrich, P. Martin, G. Palmer, C. Gabay, The interleukin (IL)-1 cytokine family – balance between agonists and antagonists in inflammatory diseases, Cytokine 76 (2015) 25–37.
[19] C. Gabay, C. Lamacchia, G. Palmer, IL-1 pathways in inflammation and human diseases, Nat. Rev. Rheumatol. 6 (2010) 232–241.
[20] C. Gabay, J.E. Towne, Regulation and function of interleukin-36 cytokines in homeostasis and pathological conditions, J. Leukocyte Biol. 97 (2015) 645–652.
[21] M. Bachmann, P. Scheiermann, L. Hardle, J. Pfeilschifter, H. Muhl, IL-36gamma/IL-1F9, an innate T-bet target in myeloid cells, J. Biol. Chem. 287 (2012) 41684–41696.
[22] A. Nerlich, N. Ruangkiattikul, K. Laarmann, N. Janze, O. Dittrich-Breiholz, M. Kracht, et al., C/EBPbeta is a transcriptional key regulator of IL-36alpha in murine macrophages, Biochim. Biophys. Acta 1849 (2015) 966–978.
[23] S. Vigne, G. Palmer, C. Lamacchia, P. Martin, D. Talabot-Ayer, E. Rodriguez, et al., IL-36R ligands are potent regulators of dendritic and T cells, Blood 118 (2011) 5813–5823.
[24] S. Vigne, G. Palmer, P. Martin, C. Lamacchia, D. Strebel, E. Rodriguez, et al., IL-36 signaling amplifies Th1 responses by enhancing proliferation and Th1 polarization of naive CD4+ T cells, Blood 120 (2012) 3478–3487.
[25] S. Mutamba, A. Allison, Y. Mahida, P. Barrow, N. Foster, Expression of IL-1Rrp2 by human myelomonocytic cells is unique to DCs and facilitates DC maturation by IL-1F8 and IL-1F9, Eur. J. Immunol. 42 (2012) 607–617.
[26] A.M. Foster, J. Baliwag, C.S. Chen, A.M. Guzman, S.W. Stoll, J.E. Gudjonsson, et al., IL-36 promotes myeloid cell infiltration, activation, and inflammatory activity in skin, J. Immunol. 192 (2014) 6053–6061.
[27] S. Frey, A. Derer, M.E. Messbacher, D.L. Baeten, S. Bugatti, C. Montecucco, et al., The novel cytokine interleukin-36alpha is expressed in psoriatic and rheumatoid arthritis synovium, Ann. Rheum. Dis. 72 (2013) 1569–1574.
[28] D. Magne, G. Palmer, J.L. Barton, F. Mezin, D. Talabot-Ayer, S. Bas, et al., The new IL-1 family member IL-1F8 stimulates production of inflammatory mediators by synovial fibroblasts and articular chondrocytes, Arthrit. Res. Ther. 8 (2006) R80.

[29] Y. Carrier, H.L. Ma, H.E. Ramon, L. Napierata, C. Small, M. O'Toole, et al., Inter-regulation of Th17 cytokines and the IL-36 cytokines in vitro and in vivo: implications in psoriasis pathogenesis, J. Invest. Dermatol. 131 (2011) 2428–2437.
[30] L.H. Lian, K.A. Milora, K.K. Manupipatpong, L.E. Jensen, The double-stranded RNA analogue polyinosinic-polycytidylic acid induces keratinocyte pyroptosis and release of IL-36gamma, J. Invest. Dermatol. 132 (2012) 1346–1353.
[31] N. Segueni, S. Vigne, G. Palmer, M.L. Bourigault, M.L. Olleros, D. Vesin, et al., Limited contribution of IL-36 versus IL-1 and TNF pathways in host response to mycobacterial infection, PLoS One 10 (2015) e0126058.
[32] L. Bozoyan, A. Dumas, A. Patenaude, L. Vallieres, Interleukin-36gamma is expressed by neutrophils and can activate microglia, but has no role in experimental autoimmune encephalomyelitis, J. Neuroinflammation 12 (2015) 173.
[33] Y. Osugi, S. Vuckovic, D.N. Hart, Myeloid blood CD11c(+) dendritic cells and monocyte-derived dendritic cells differ in their ability to stimulate T lymphocytes, Blood 100 (2002) 2858–2866.
[34] A. Johnston, X. Xing, A.M. Guzman, M. Riblett, C.M. Loyd, N.L. Ward, et al., IL-1F5, -F6, -F8, and -F9: a novel IL-1 family signaling system that is active in psoriasis and promotes keratinocyte antimicrobial peptide expression, J. Immunol. 186 (2011) 2613–2622.
[35] L.C. Zaba, J. Fuentes-Duculan, R.M. Steinman, J.G. Krueger, M.A. Lowes, Normal human dermis contains distinct populations of CD11c+BDCA-1+ dendritic cells and CD163+FXIIIA+ macrophages, J. Clin. Invest. 117 (2007) 2517–2525.
[36] C.E. Angel, E. George, A.E. Brooks, L.L. Ostrovsky, T.L. Brown, P.R. Dunbar, Cutting edge: CD1a+ antigen-presenting cells in human dermis respond rapidly to CCR7 ligands, J. Immunol. 176 (2006) 5730–5734.
[37] F.O. Nestle, X.G. Zheng, C.B. Thompson, L.A. Turka, B.J. Nickoloff, Characterization of dermal dendritic cells obtained from normal human skin reveals phenotypic and functionally distinctive subsets, J. Immunol. 151 (1993) 6535–6545.
[38] D. Dietrich, C. Gabay, Inflammation: IL-36 has proinflammatory effects in skin but not in joints, Nat. Rev. Rheumatol. 10 (2014) 639–640.
[39] H. Blumberg, H. Dinh, C. Dean Jr., E.S. Trueblood, K. Bailey, D. Shows, et al., IL-1RL2 and its ligands contribute to the cytokine network in psoriasis, J. Immunol. 185 (2010) 4354–4362.
[40] H. Blumberg, H. Dinh, E.S. Trueblood, J. Pretorius, D. Kugler, N. Weng, et al., Opposing activities of two novel members of the IL-1 ligand family regulate skin inflammation, J. Exp. Med. 204 (2007) 2603–2614.
[41] K.A. Milora, H. Fu, O. Dubaz, L.E. Jensen, Unprocessed interleukin-36alpha regulates psoriasis-like skin inflammation in cooperation with interleukin-1, J. Invest. Dermatol. 135 (2015) 2992–3000.
[42] M. Friedrich, C. Tillack, A. Wollenberg, J. Schauber, S. Brand, IL-36gamma sustains a proinflammatory self-amplifying loop with IL-17C in anti-TNF-induced psoriasiform skin lesions of patients with Crohn's disease, Inflamm. Bowel Dis. 20 (2014) 1891–1901.
[43] K.J. Mylonas, M.G. Nair, L. Prieto-Lafuente, D. Paape, J.E. Allen, Alternatively activated macrophages elicited by helminth infection can be reprogrammed to enable microbial killing, J. Immunol. 182 (2009) 3084–3094.
[44] D. Duluc, M. Corvaisier, S. Blanchard, L. Catala, P. Descamps, E. Gamelin, et al., Interferon-gamma reverses the immunosuppressive and protumoral properties and prevents the generation of human tumor-associated macrophages, Int. J. Cancer 125 (2009) 367–373.
[45] C. Guiducci, A.P. Vicari, S. Sangaletti, G. Trinchieri, M.P. Colombo, Redirecting in vivo elicited tumor infiltrating macrophages and dendritic cells towards tumor rejection, Cancer Res. 65 (2005) 3437–3446.
[46] F. Porcheray, S. Viaud, A.C. Rimaniol, C. Leone, B. Samah, N. Dereuddre-Bosquet, et al., Macrophage activation switching: an asset for the resolution of inflammation, Clin. Exp. Immunol. 142 (2005) 481–489.
[47] A. Sica, A. Mantovani, Macrophage plasticity and polarization: in vivo veritas, J. Clin. Invest. 122 (2012) 787–795.
[48] C. Lamacchia, G. Palmer, E. Rodriguez, P. Martin, S. Vigne, C.A. Seemayer, et al., The severity of experimental arthritis is independent of IL-36 receptor signaling, Arthrit. Res. Ther. 15 (2013) R38.